

Figure 1

Human Mature DNase I

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      10      20      30      40      50
LKIAAFNIQTFGETKMSNATLVSYIVQILSRVDIALVQEVDRDShLTAVGK
      60      70      80      90     100
LLDNLNQDAPDTYHYVVSEPLGRNSYKERYLFVYRPDQVSAVDSYYYDDG
     110     120     130     140     150
CEPCGNDTFNREPAIVRFFSRFTEVREFAIVPLHAAPGDVAEIDALYDV
     160     170     180     190     200
YLDVQEKWGLEDVMLMGDFNAGCSYVRPSQWSSIRLWTSPTFQWLIPDSA
     210     220     230     240     250
DTTATPTHCAVDRIVVAGMLLRGAVVPDSALPFNFQAAYGLSDQLAQAI
     260
DHYPVEVMLK
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Figure 2

Plasmid DNA Digestion Assays

DNase I Variants	Linear DNA Digestion Assay	Supercoiled DNA Digestion Assay	
	Relative Linear DNA Digestion Activity	L/R ratio	Relative Nicking Activity
native human DNase I	1.0 ± 0.1	1.0	1.0 ± 0.0
Q9R	3.5 ± 0.4	2.3	3.4 ± 0.5
E13K	3.9 ± 0.1		
E13R	6.0 ± 0.5	5.4	2.2 ± 0.0
T14K	4.2 ± 0.1	4.7	2.9 ± 0.8
T14R	3.5 ± 0.7		
H44K	2.0 ± 0.4	2.3	1.8 ± 0.3
H44R	3.6 ± 0.5		
N74K	6.0 ± 0.1	4.7	7.3 ± 1.0
N74R	4.1 ± 0.8		
S75K	1.5 ± 0.2		
T205K	4.7 ± 0.2	5.4	2.8 ± 0.7
T205R	2.3 ± 0.3		
E13R:N74K	26.7 ± 4.1	12.3	6.9 ± 1.6
Q9R:E13R:N74K	38.3 ± 1.2	16.5	6.3 ± 2.2
E13R:N74K:T205K	19.5 ± 6.4		
Q9R:E13R:N74K:T205K	30.5 ± 7.5		

All data is normalized to native human DNase I.

Figure 3

DNA Hyperchromicity Assay

DNase I Variants	$1/K_m$	V_{max}	V_{max}/K_m
native human DNase I	1.0 ± 0.1	1.0 ± 0.1	1.0
Q9R	0.9 ± 0.2	2.8 ± 0.4	2.6
E13K	2.5 ± 0.4	1.8 ± 0.1	4.5
E13R	4.3 ± 1.4	1.5 ± 0.1	6.5
T14K	2.3 ± 0.9	1.1 ± 0.2	2.5
T14R	2.1 ± 0.8	0.7 ± 0.1	1.5
H44K	2.3 ± 0.5	1.1 ± 0.1	2.5
H44R	1.7 ± 0.2	1.0 ± 0.1	1.7
N74K	0.4 ± 0.2	5.5 ± 1.3	2.3
N74R	2.6 ± 0.8	3.1 ± 0.3	8.1
S75K	18.5 ± 2.0	0.4 ± 0.1	7.4
T205K	2.4 ± 0.8	2.1 ± 0.4	5.0
T205R	3.0 ± 1.2	1.0 ± 0.1	3.0
E13R:N74K	5.0 ± 1.7	5.3 ± 0.5	26.5
Q9R:E13R:N74K	4.9 ± 1.3	7.0 ± 0.4	34.3
E13R:N74K:T205K	5.0 ± 1.9	6.3 ± 0.6	31.5
Q9R:E13R:N74K:T205K	5.6 ± 1.4	3.8 ± 0.3	21.3

All data is normalized to native human DNase I.

Figure 4

Effect of NaCl on Human DNase I Variants

